Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 555

Product Details	
Size	1 mg
Species	Mouse
Published Species	Mouse
Expression System	Goat / IgG
Class	Polyclonal
Type	Secondary Antibody
Conjugate	Alexa Fluor® 555
Immunogen	Gamma Immunoglobins Heavy and Light chains
Form	Liquid
Concentration	2 mg/mL
Purification	purified
Storage buffer	PBS, pH 7.5
Contains	5mM sodium azide
Storage Conditions	4° C, store in dark
RRID	AB_2535844

Applications	Tested Dilution	Publications
Flow Cytometry (Flow)	1-10 µg/mL	2 Publications
Immunocytochemistry (ICC)	2 µg/mL	20 Publications
Immunofluorescence (IF)	2 µg/mL	2 Publications
Immunohistochemistry (IHC)	1-10 µg/mL	18 Publications
Immunohistochemistry (Paraffin) (IHC (P))	-	3 Publications
Immunohistochemistry - Free Floating (IHC (Free))	-	1 Publication
Miscellaneous PubMed (Misc)	-	73 Publications
Western Blot (WB)	-	3 Publications

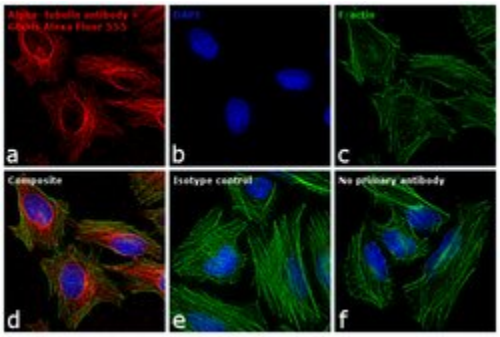
Product Specific Information

To minimize cross-reactivity, these goat anti-mouse IgG (H+L) whole secondary antibodies have been affinity purified and cross-adsorbed against human IgG and human serum prior to conjugation. Cross-adsorption or pre-adsorption is a purification step to increase specificity of the antibody resulting in higher sensitivity and less background staining. The secondary antibody solution is passed through a column matrix containing immobilized serum proteins from potentially cross-reactive species. Only the nonspecific-binding secondary antibodies are captured in the column, and the highly specific secondaries flow through. The benefits of this extra step are apparent in multiplexing/multicolor-staining experiments (e.g., flow cytometry) where there is potential cross-reactivity with other primary antibodies or in tissue/cell fluorescent staining experiments where there are may be the presence of endogenous immunoglobulins.

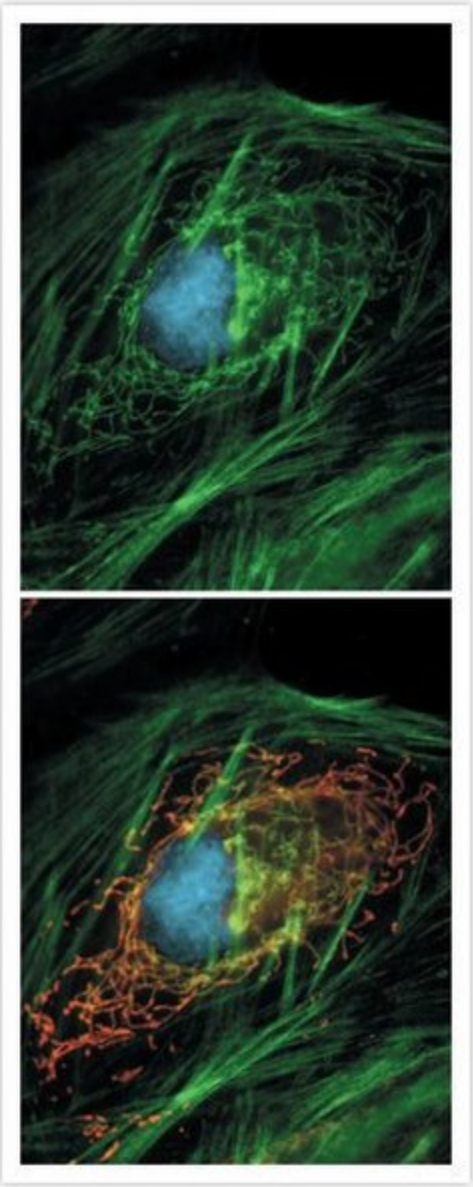
Alexa Fluor dyes are among the most trusted fluorescent dyes available today. Invitrogen™ Alexa Fluor 555 dye is a bright, orange-fluorescent dye with excitation ideally suited to the 555 nm laser line. For stable signal generation in imaging and flow cytometry, Alexa Fluor 555 dye is pH-insensitive over a wide molar range. Probes with high fluorescence quantum yield and high photostability allow detection of low-abundance biological structures with great sensitivity. Alexa Fluor 555 dye molecules can be attached to proteins at high molar ratios without significant self-quenching, enabling brighter conjugates and more sensitive detection. The degree of labeling for each conjugate is typically 2-8 fluorophore molecules per IgG molecule; the exact degree of labeling is indicated on the certificate of analysis for each product lot.

Using conjugate solutions: Centrifuge the protein conjugate solution briefly in a microcentrifuge before use; add only the supernatant to the experiment. This step will help eliminate any protein aggregates that may have formed during storage, thereby reducing nonspecific background staining. Because staining protocols vary with application, the appropriate dilution of antibody should be determined empirically. For the fluorophore-labeled antibodies a final concentration of 1-10 µg/mL should be satisfactory for most immunohistochemistry and flow cytometry applications.

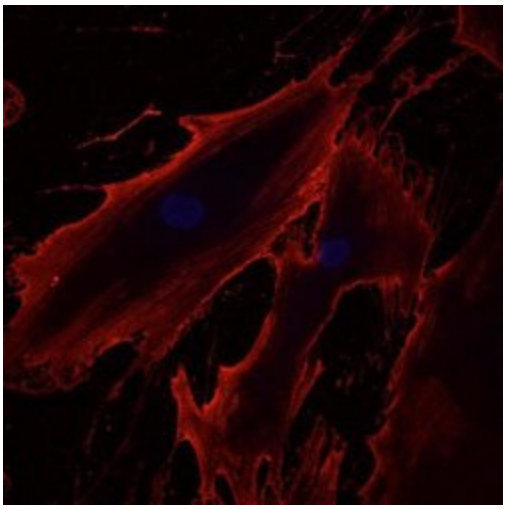
Product Images For Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 555



Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody (A-21422) in IF
Immunofluorescence analysis of Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor® 555 conjugate was performed using HeLa cells stained with alpha Tubulin (236-10501) Mouse Monoclonal Antibody (Product # A11126). The cells were fixed with 4% paraformaldehyde for 10 minutes, permeabilized with 0.1% Triton™ X-100 for 10 minutes, blocked with 1% BSA for 1 hour and labeled with 2 µg/mL primary antibody for 3 hours at room temperature. Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor® 555 (Product # A-21422) was used at a concentration of 2 µg/mL in phosphate buffered saline containing 0.2% BSA for 45 minutes at room temperature, for detection of alpha Tubulin in the cytoplasm (Panel a: red). Nuclei (Panel b: blue) were stained with DAPI in SlowFade® Gold Antifade Mountant (Product # S36938). F-actin was stained with Alexa Fluor® 488 Phalloidin (Product # A12379), 1:300) (Panel c: green). Panel d represents the composite image. No nonspecific staining was observed with the secondary antibody alone (panel f), or with an isotype control (panel e). The images were captured at 60X magnification.



Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody (A-21422) in IF
HeLa cell transfected with pShooter pCMV/myc/mito/GFP, then fixed and permeabilized. Green-fluorescent protein (GFP) localized in the mitochondria was labeled with anti-GFP mouse IgG_{2a} (Product # A-11120) and detected with orange-fluorescent Alexa Fluor® 555 goat anti-mouse IgG (Product # A-21422), which colocalized with the dim GFP fluorescence. F-actin was labeled with green-fluorescent Alexa Fluor® 488 phalloidin (Product # A12379), and the nucleus was stained with blue-fluorescent DAPI (Product # D1306, D3571, D21490). The sample was mounted using ProLong® Gold antifade reagent (Product # P36930). Some GFP fluorescence is retained in the mitochondria after fixation (top), but immunolabeling and detection greatly improve visualization (bottom).



Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody (A-21422) in IF
Immunofluorescent analysis of CD44 (red) in human bone marrow derived mesenchymal stem cells. The cells were fixed with 2% paraformaldehyde, and blocked with 2% normal donkey serum diluted in PBS for 30 minutes at room temperature. Cells were stained with a CD44 monoclonal antibody (Product # MA5-12394) at a dilution of 10 µg/mL in blocking buffer for 1 hour at room temperature, and then incubated with a Goat anti-mouse IgG (H+L) Secondary Antibody, Alexa Fluor® 555 conjugate (Product # A-21422) at a dilution of 1:800 for 1 hour at room temperature (red). Nuclei (blue) were stained with Hoechst 33342 dye (Product # 62249). Note: Data courtesy of Innovators Program.

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122 References

Immunocytochemistry (20)

Biological procedures online

Effective Visualization and Easy Tracking of Extracellular Vesicles in Glioma Cells.

"A-21422 was used in Immunocytochemistry-immunoflourescence to report a new method developed by us for visualizing EVs using simple immune-fluorescence based technique, wherein the isolated EVs can be stained with fluorescently tagged antibodies to proteins present in EVs."

Authors: Mondal A,Ashiq KA,Phulpagar P,Singh DK,Shiras A

Species
Mouse
Not Applicable

Dilution
Not Cited
Not Cited

Year
2020

Frontiers in oncology

Biglycan Regulates MG63 Osteosarcoma Cell Growth Through a LPR6/-Catenin/IGFR-IR Signaling Axis.

"A-21422 was used in Immunocytochemistry-immunoflourescence to report a novel mechanism where biglycan through a LRP6/-catenin/IGF-IR signaling axis enhances osteosarcoma cell growth."

Authors: Aggelidakis J,Berdiaki A,Nikitovic D,Papoutsidakis A,Papachristou DJ,Tsatsakis AM,Tzanakakis GN

Species
Mouse
Not Applicable

Dilution
Not Cited
Not Cited

Year
2020

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Miscellaneous PubMed (73)

Biological procedures online

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Authors: Mondal A,Ashiq KA,Phulpagar P,Singh DK,Shiras A

Species
Mouse
Not Applicable

Dilution
Not Cited
Not Cited

Year
2020

Frontiers in neurology

Cytokine Levels in Inner Ear Fluid of Young and Aged Mice as Molecular Biomarkers of Noise-Induced Hearing Loss.

"A-21422 was used in Immunohistochemistry to show the inner ear fluid of mice with sensorineural hearing loss show increased L-6, TNF-, and CXCL1 cytokine levels."

Authors: Landegger LD,Vasilijic S,Fujita T,Soares VY,Seist R,Xu L,Stankovic KM

Species
Not Applicable

Dilution
1:1000

Year
2020

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IHC (18) IHC (P) (3) WB (3) Flow (2) IF (2) IHC (Free) (1)